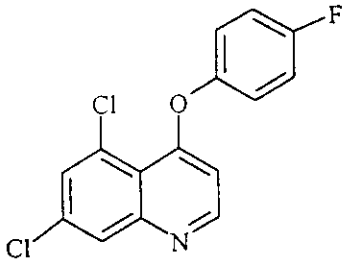
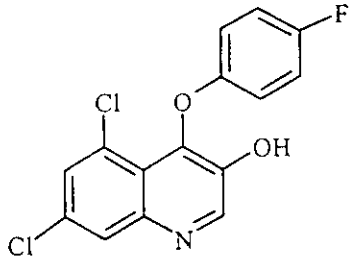
INTRODUCTION

Quinoxyfen [5,7-dichloro-4-(4-fluorophenoxy)quinoline] is a fungicide active against powdery mildew in cereals, vegetables, grapes, stone fruits, and other crops. The major mode of action at the cellular level (in powdery mildew) involves the inhibition of primary appressorial formation. Quinoxyfen is proposed for use in grape and hops in the United States at a maximum seasonal rate of 600 g ai/ha.



Quinoxyfen
CAS No. 124495-18-7



Quinoxyfen 3-OH
CAS No. Unavailable

Method ERC 94.27 was developed and validated for the determination of residues of quinoxyfen and its major metabolite, quinoxyfen 3-OH, in soil (1). This report contains the method validation recovery data that were obtained to determine the accuracy, precision, specificity, and ruggedness of the method.

EXPERIMENTAL

Sample Origin, Numbering, and Preparation

Untreated soil samples were obtained for use as control samples. The numbers, origins, and soil types of the control samples are contained in Table 1 in Appendix A.

The control soil samples were sieved through either a 4-mm or 2.36-mm sieve to produce a finely divided sample.

Fortification of Recovery Samples

As described in Method ERC 94.27 (Appendix A), nominal 5-g samples of untreated control soil (Step 6.7.1) were fortified with the mixed spiking solution for the generation of method validation recovery data. Reagent blank and control samples receiving no fortification were also prepared for analysis with the recovery samples to check for background interferences.

Sample Preparation, Extraction, and Analysis

Samples were prepared and analyzed as described in Section 6.7 in the method for the determination of quinoxyfen and quinoxyfen 3-OH in soil (ERC 94.27, Appendix A).

Residues of quinoxyfen and the quinoxyfen 3-OH metabolite were extracted from soil using an 80% acetone/20% 0.1 N hydrochloric acid solution. After addition of sodium bicarbonate solution to the extract, both analytes were partitioned into hexane which was then evaporated to dryness. The residuum was then methylated and both analytes were extracted from the aqueous layer into methyl-*tert*-butyl ether. The ether layer was evaporated to dryness and the residuum was reconstituted in hexane prior to an aminopropyl solid-phase extraction using 5% acetone in hexane to elute both the quinoxyfen and the methylated metabolite. The eluate is evaporated to dryness and reconstituted in 0.1% corn oil in isoctane containing 0.2 µg/mL 1,4-dibromonapthalene as an internal standard. Quinoxyfen and the methylated metabolite were quantified by gas chromatography with mass selective detection (GC-MSD).

Analytical Instrumentation

Samples were analyzed using the GC-MSD instrumental operating conditions that are described in Section 6.4 of analytical method ERC 94.27 (Appendix A).

Calculations

Calculation of percent recovery for fortified samples was performed as described in Section 7 of analytical method ERC 94.27 (Appendix A)

Statistical Treatment of Data

Statistical treatment of the data included calculation of means, standard deviations, and percent relative standard deviations of the results for the fortified recovery samples.

Study Personnel

A. Gambie was the Study Director of the method validation study, and A. Gambie and S. Wood were the authors of Method ERC 94.27. The method validation report was authored by E. L. Olberding.

DowElanco Europe
Letcombe Laboratory
Letcombe Regis
Wantage
Oxon. OX12 9JT
Telephone: 01235 772900
Fax: 01235 774803

DowElanco

Effective: 7 Jun 1995
Supercedes : New

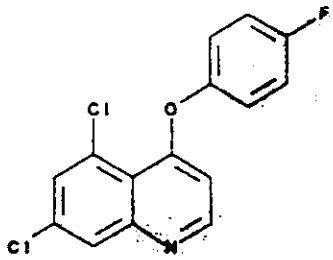
ERC 94.27

Determination of XDE-795 and the 3-Hydroxy Metabolite Residues in Soil

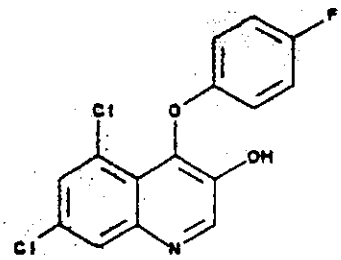
Authors : A Gambie, S Wood

1. SCOPE

This method is applicable to the quantitative determination of XDE-795* and a metabolite, 3-hydroxy XDE-795** residues in soil down to lowest validated levels of 0.01 mg/kg. The method has been validated by the analysis of untreated and fortified samples for residues of XDE-795 and 3-hydroxy XDE-795 in soil over the range 0.01 - 1.0 mg/kg.



XDE-795



3-hydroxy XDE-795

2. PRINCIPLE

XDE-795 and 3-hydroxy XDE-795 are extracted from the soil by shaking with acidic acetone. After addition of sodium bicarbonate solution to the extract, both analytes are partitioned into hexane which is then evaporated to dryness. The 3-hydroxy XDE-795 in the residuum is then methylated and both analytes are

* Experimental name for : 5,7-dichloro-4-(*p*-fluorophenoxy)-quinoline (IUPAC)
**Experimental name for : 3-hydoxy-5,7-dichloro-4-(*p*-fluorophenoxy)-quinoline (IUPAC)

extracted from the aqueous layer into methyl tertiary butyl ether. The ether layer is evaporated to dryness and the residuum is reconstituted in hexane prior to an aminopropyl solid phase extraction using 5% acetone in hexane to elute both XDE-795 and the methylated metabolite. The eluate is evaporated to dryness and reconstituted in 0.1% corn oil in tri-methyl pentane containing 0.2 µg/mL, 1,4-dibromonapthalene as an internal standard. XDE-795 and the methylated metabolite are quantified by gas chromatography with mass selective detection.

3. PRODUCT SAFETY PRECAUTIONS

Each analyst should be acquainted with potential hazards of the reagents, products and solvents before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE AND OTHER INTERNALLY- GENERATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents, reactants and solvents must be in compliance with the appropriate government regulations.

4. EQUIPMENT*

4.1 Laboratory Equipment

4.1.1 Sieves : 4, 2.36 mm - Fisons Scientific Equipment Ltd.

4.1.2 MSE model GF-8 centrifuge fitted with a six place rotor and 380mL slotted cups with rubber cushions - Fisons Scientific Equipment Ltd.

4.1.3 Reciprocating shaker model LS 20 (150 rpm) - Gerhardt UK Ltd.

4.1.4 Polytron sample homogeniser, model PT 3000 - Philip Harris Scientific.

4.1.5 Techne sample concentrator model SC3 - Fisons Scientific Equipment Ltd.

4.1.6 Ultrasonic bath - Decon FS100 - Fisons Scientific Equipment Ltd.

4.1.7 Vacuum manifold apparatus for solid-phase extraction - Jones Chromatography Ltd.

4.1.8 Vortex machine IKA Vibrox - Fisons Scientific Equipment Ltd.

ERC 94.27

4.2 Chromatographic System

4.2.1 Gas chromatograph: Hewlett Packard 5890 fitted with a 7673A autosampler and a 5970 Mass Selective Detector, or equivalent.

4.2.2 Data Handling - HP Chem Station G1034C

4.2.3 Column - HP-Ultra 2 (5% phenyl methyl silicone) 12.5m x 0.2mm i.d. x 0.3µm film thickness. - Hewlett Packard Ltd.

4.3 Laboratory Glassware and Plasticware

4.3.1 Volumetric glassware : pipettes (1.0 - 20.0mL), flasks (100.0mL - 250mL), measuring cylinders (50 - 1000mL) - Fisons Scientific Equipment Ltd.

4.3.2 8oz (200mL) and 4oz glass jars and caps - Bristol Bottle Co. Ltd.

4.3.3 8 dram (30mL) screw cap vials - Fisons Scientific Equipment Ltd.

4.3.4 11 dram (50mL) screw cap vials - Fischer Scientific Equipment

4.3.5 2.0mL, 5.0mL and 10mL disposable pipettes - Fisons Scientific Equipment Ltd.

4.3.6 2mL gas chromatography vials and aluminium/red rubber vial caps - Owen Polyscience Ltd.

4.3.7 2mL plastic disposable transfer pipettes - Fisons Scientific Equipment Ltd.

4.3.8 Unicaps R3 plastic screw top to fit 8 dram and 11 dram vials and caps for 8oz jars - Bristol Bottle Co. Ltd.

*The full address of all suppliers named above is included in Appendix 1.

ERC 94.27

5. MATERIALS AND REAGENTS*

5.1 Materials

5.1.1 Acetone - Distol grade - Fisons Scientific Equipment Ltd.

5.1.2 Hexane - Distol grade - Fisons Scientific Equipment Ltd.

5.1.3 Tri-methyl pentane - HPLC grade - Fisons Scientific Equipment Ltd.

5.1.4 Methyl tertiary butyl ether - HPLC grade - Fisons Scientific Equipment Ltd.

5.1.5 Toluene - Distol grade - Fisons Scientific Equipment Ltd.

5.1.6 Water - HPLC grade - Fisons Scientific Equipment Ltd.

5.1.7 Tetrahydrofuran - Distol grade - Fisons Scientific Equipment Ltd.

5.1.8 Hydrochloric acid (concentrated) - AnalaR - Fisons Scientific Equipment Ltd.

5.1.9 Sodium bicarbonate - AnalaR - Fisons Scientific Equipment Ltd.

5.1.10 Tetrabutyl ammonium hydroxide - 0.1M (phosphate buffered) - Fisons Scientific Equipment Ltd.

5.1.11 Ammonia solution (S.G. 0.88) - Primar grade - Fisons Scientific Equipment Ltd.

5.1.12 Iodomethane, 99% - Aldrich Chemical Co Ltd

5.1.13 Helium (GC carrier gas) - MG Gas Products Ltd.

5.1.14 Nitrogen (GC auxillary gas) - Nitrogen generator NG 750 - Nitrox Ltd.

5.1.15 Dry ice pellets - MG Gas Products Ltd.

5.1.16 Aminopropyl solid phase extraction cartridge, 500mg (Isolute) - Jones Chromatography Ltd.

5.1.17 Corn oil - Mazola - CPC (UK) Ltd.

5.1.18 Analytical standard of XDE-795 and 3-hydroxy XDE-795 available from Analytical Standards Co-ordinator, Research and Development Centre, DowElanco Europe

5.1.19 1,4-dibromonaphthalene - Phase Separation Ltd.

*The full address of all suppliers named above is included in Appendix 1.

ERC 94.27

5.2 Reagents

5.2.1 1% (v/v) water in tetrahydofuran (THF)

5.2.2 0.12 M hydrochloric acid: 10 mL hydrochloric acid diluted to 1L with HPLC grade water.

5.2.3 Extraction solvent - acetone/0.12 M hydrochloric acid 80:20 (v/v).

5.2.4 Sodium bicarbonate solution - 5% (w/v) aqueous solution.

5.2.5 Elution solvent - 5% (v/v) Acetone/Hexane.

6. PROCEDURE

6.1 Stock and Fortification Solutions

6.1.1 Dissolve 100mg of analytical standard of XDE-795 in 100.0mL of acetone. Make dilutions of this 1000 µg/mL stock solution with acetone to give 100, 10, 1 and 0.1 µg/mL solutions for use in recovery determinations.

6.1.2 Dissolve 50mg of analytical standard of 3-hydroxy XDE-795 in 250.0mL of THF/1%water. Make dilutions of this 200 µg/mL stock solution with acetone to give 10, 1 and 0.1 µg/mL solutions for use in recovery determinations.

6.1.3 Prepare a stock solution of 3-MeO XDE-795 by methylating 5mL of the 200 µg/mL stock solution of 3-hydroxy XDE-795 according to steps 6.7.8 - 6.7.9. Make up the 3-MeO XDE-795 solution to 100mL in tri-methylpentane (TMP) to give a stock solution of 10 µg/mL for use in making calibration solutions.

6.2 Internal Standard Solutions

Dissolve 100mg of 1,4-dibromonaphthalene in 100.0mL TMP. Make dilutions of this 1000 µg/mL stock solution with 0.1% corn oil in tri-methyl pentane to give 10 µg/mL and 0.2 µg/mL solutions.

6.3 Calibration Solutions

Prepare solutions of XDE-795 and 3-MeO XDE-795 in 0.1% corn oil in TMP from the 1000 µg/mL stock solution (Section 6.1.1) and the 10 µg/mL stock solution (Section 6.1.3) over the range 0.01 - 1.0 µg/mL. To each solution also add an appropriate aliquot of the 10 µg/mL 1,4-dibromonaphthalene to give a final concentration of 0.2 µg/mL in each solution. Run under the chromatographic conditions listed in Section 6.4. Plot peak area response ratio against concentration to establish the linearity of the detector. A typical calibration plot is shown in Figure 1.

ERC 94.27

6.4 Chromatographic Conditions (Note 9.1)

Column	:	12.5m x 0.2mm i.d. 0.3µm film thickness HP-Ultra 2 (5% phenyl methyl silicone).
Injection mode	:	Splitless
Purge Information	:	Valve off at 0 min Valve on at 0.75 min
Carrier gas	:	Helium (head pressure 50 kPa)
Septum purge gas	:	2 - 3 mL/minute
Oven temperature	:	60°C for 1 minute then 20°C/minute ramp to 220°C for 2 minutes increase at 20°C/min to 260°C, hold for 2 minutes Post run at 300°C for 3 minutes.
Injector temperature	:	300°C
Detector	:	Mass selective detector - EM voltage at autotune value. - ions monitored : 1,4-dibromonaphthalene; 286 amu XDE-795; 237 and 272 amu 3-MeO XDE-795; 337 and 330 amu
Injection volume	:	2µL
Retention time (typical)	:	1. 1,4-dibromonaphthalene : 7.1 minutes 2. XDE-795 : 10.2 minutes 3. 3-MeO XDE-795 : 13.2 minutes
Quantitation	:	Peak Area
Total Analysis Time	:	Approximately 19 minutes

Typical chromatograms are shown in Figure 6.

ERC 94.27

6.5 Sample Preparation

6.5.1 Soil is laid out to dry such that it can be sieved through either a 4 mm or 2.36mm sieve to produce a finely divided sample.

6.6 Method Validation

Validate the analytical procedure given in Section 6.7 by analysing the following:

At least three untreated samples (in duplicate).

At least three untreated samples after fortification at the lowest validation level (in duplicate). The lowest validation level is defined as "at least 4 times the average untreated value". At least one sample (in duplicate) fortified at an intermediate level and one sample at a level exceeding the expected maximum residue found (in duplicate).

6.7 Sample and Fortified Sample Analysis

Include a reagent blank and procedural recovery in each analytical batch. Analyse all treated and untreated samples in duplicate.

6.7.1 Weigh duplicate 5g ± 0.10g dry weight equivalent portions of the sample into 8 dram vials (Note 9.3). Add the required volume of the appropriate fortification solution to the recovery samples.

6.7.2 Add 10mL of acetone:0.12M HCl (80:20 v/v) to each jar and shake for 15 minutes on a reciprocating shaker, then centrifuge the sample at 2000 rpm for 5 minutes. Transfer the supernatant to a 4oz. jar.

6.7.3 Repeat extraction twice more with 10 mL of acetone:0.12M HCl (80:20 v/v) combining the supernatants.

6.7.4 Add 60 mL of 5% (w/v) sodium bicarbonate solution and 20 mL hexane.

6.7.5 Shake for 15 minutes, centrifuge for 5 minutes at 2000 rpm.

6.7.6 Transfer the hexane into a 8 dram vial. Repeat with another 20 mL of hexane combining the extracts.

6.7.7 Place the vial in a heating block set at 40°C and evaporate the hexane to dryness under a gentle stream of nitrogen.

ERC 94.27

6.7.8 Prepare a new batch standard with each batch of samples. Transfer 200µL of the 100µg/mL fortification solution containing XDE-795, and 100µL of the 200 µg/mL fortification solution containing 3-hydroxy XDE-795 to an 8 dram vial, evaporate the solvent and proceed with methylation step below concurrent with the analytical batch.

6.7.9 Methylate the extract according to the following procedure. Add 250µL 0.1M tetrabutyl ammonium hydroxide, 250µL ammonia solution, 1mL toluene and 250µL iodomethane. Cap and vortex at 1400 rpm for 90 minutes. Add 10mL water and 10mL tertiary methyl butyl ether shake for 5 minutes and centrifuge at 2000 rpm for 2 minutes. Repeat with another 10mL of ether and combine the extracts. In the case of the analytical batch standard, repeat the extraction with another 10mL of tertiary methyl butyl ether. Transfer the ether layer to an 8 dram vial and evaporate to dryness in a heating block set at 40°C under a gentle stream of nitrogen.

6.7.10 Reconstitute the residuum in 5mL of hexane and sonicate for 1 minute. In the case of the batch standard, reconstitute in 0.1% corn oil in tri-methyl pentane containing 0.2 µg/mL 1,4-dibromonapthalene as an internal standard, quantitatively transfer to a 100mL volumetric flask and make up to volume. This will give a batch standard of 0.2 µg/mL of XDE-795 and 3-MeO XDE-795.

6.7.11 Prepare an aminopropyl solid phase extraction cartridge by conditioning with 10mL of hexane.(Note 9.2)

6.7.12 Add the extract from step 6.7.10 to the cartridge discarding the eluate.

6.7.13 Rinse the vial with a further 5mL volume of hexane and use this to wash the cartridge, discarding the eluate.

6.7.14 Elute the analytes with 6 mL of 5%v/v acetone/hexane collecting the eluate in an 8 dram vial.

6.7.15 Place the vial in a heating block at 40°C and evaporate the eluate to dryness under a gentle stream of nitrogen.

6.7.16 Reconstitute the residuum in 1 mL 0.1% corn oil in TMP containing 0.2 µg/mL 1,4-dibromonapthalene as an internal standard and sonicate for 1 minute .

6.7.17 Chromatograph samples using the conditions given in Section 6.4, injecting a standard solution between every two samples.

6.7.18 For sample extracts which contain analyte concentrations of greater than 1.0 µg/mL, dilute with the internal standard solution as in step 6.7.16 to give an analyte concentration in the range 0.01 -1.0 µg/mL.

ERC 94.27

7 CALCULATIONS

7.1 Calculation of XDE-795 and 3-Hydroxy Metabolite Residues

The XDE-795 and 3-hydroxy metabolite concentration are calculated by an internal standard method using the following equation :

$$\text{mg/kg} = \frac{\text{Average Sample Response Ratio}}{\text{Average Standard Response Ratio}} \times \frac{A \times B \times C \times D}{E}$$

where :

$$\text{Response ratio} = \frac{\text{Peak Area Quantitation ion}}{\text{Peak Area 1,4 dibromonaphthalene (286 amu)}}$$

A = concentration of standard, µg/mL

B = final volume (mL), soil = 1 mL

C = procedural dilution factor

D = additional dilution factor, if applicable

E = initial sample weight (g), soil = 5g(Note 9.3)

Quantitation ions; XDE-795 = 237 amu
3-MeO-XDE-795 = 337 amu

Confirmation of the presence of XDE-795 at concentrations greater than the lowest validated concentration can be made when the ratio of the 272 and 237 amu ions in the sample is within 10% of the mean of the ratio of these ions in the bracketing standards. (Note 9.4)

Confirmation of the presence of 3-Methoxy XDE-795 at concentrations greater than the lowest validated concentration can be made when the ratio of the 320 and 337 amu ions in the sample is within 10% of the mean of the ratio of these ions in the bracketing standards.

7.2 Calculation of % Recovery

Calculate the percentage recovery as follows :

$$\text{\% Recovery} = \frac{(\text{mg/kg found} - \text{mg/kg untreated}) \times 100}{\text{Fortification Level mg/kg added}}$$

ERC 94.27

9. NOTES

9.1 The gas chromatograph should be "primed" for use by injection of either sample extracts or calibration standards containing 0.1% corn oil until the system provides reproducible responses for replicate injections.

9.2 The elution profile of the solid phase cartridges should be checked in the presence of control sample extract.

9.3 The method calls for sample weight to be a dry weight equivalent. The moisture content can be determined by heating pre-weighed samples (25g) at 105°C for 16 hours and reweighing when cool.

If wet weight = w_1 and dry weight = w_2 then 5g dry weight equivalent =5x $1/\left[1-\frac{w_1-w_2}{w_1}\right]$

9.4 If a sample exhibits both the 237 and 272 amu ions at the same retention time as XDE-795 but the ratio of the ions is not within ±10% of that of the bracketing standard ,the 307 ion should also be monitored. The ratio of the 307 and 272 amu ions in the sample and the bracketing standards should then be compared. If the sample ion ratio is within ±10% of that of the bracketing standard this confirms the presence of XDE-795.

ERC 94.27

Appendix 1

Suppliers Addresses

Bristol Bottle Co. Ltd., Unit 1, Ashmead Trading Estate, Keynsham, UK.

CPC (UK) Ltd., Claygate House, Esher, Surrey, UK.

Fisher Scientific Equipment, International Division, 50 Federn Road, Springfield NJ 07081,USA

Fisons Scientific Equipment Ltd., Bishop Meadow Road, Loughborough Road, Leicestershire, UK.

Frank Driver Ltd., 206 Elgar Road, Reading, Berkshire, UK.

Gerhardt UK Ltd., Underwood Lane, Crewe, Cheshire, UK.

Glen Creston Ltd., 16 Dalston Gardens, Stanmore, Middlesex, UK.

Hewlett Packard Ltd., King Street, Wokingham, Berkshire, UK.

Jones Chromatography Ltd., New Road, Hengoed, Mid Glamorgan, UK.

MG Gas Products Ltd., Cedar House, 39 London Road, Reigate, Surrey, UK.

Nitrox Ltd., Cornwallis House, Howard Chase, Basildon, Essex, UK.

Owen Polyscience Ltd., 34 Chester Road, Macclesfield, Cheshire, UK.

Phase Separation Ltd., Deeside Industrial Park, Deeside, Clwyd, UK.

Philip Harris Scientific, 618 Western Avenue, Park Royal, London W3, UK.

Stephan Machinery (UK) Ltd., Unit 7, Felthambrook Industrial Estate, Felthambrook Way, Feltham, Middlesex, UK.